

# Preparation and Sustained-Release Property of Triblock Copolymer/Calcium Phosphate Nanocomposite as Nanocarrier for Hydrophobic Drug

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Received: 21 November 2009 / Accepted: 30 January 2010 / Published online: 14 February 2010  
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**Abstract** The P123/ACP nanocomposite with sizes less than 100 nm consisting of triblock copolymer P123 and amorphous calcium phosphate (ACP) has been prepared by using an aqueous solution containing  $\text{CaCl}_2$ ,  $(\text{NH}_4)_3\text{PO}_4$ , and P123 at room temperature. The P123/ACP nanocomposite is used as the nanocarrier for hydrophobic drug ibuprofen, based on the combined advantages of both amphiphilic block copolymer and calcium phosphate delivery system. The P123/ACP nanocomposite has a much higher ibuprofen loading capacity (148 mg/g) than the single-phase calcium phosphate nanostructures. The drug release percentage of the P123/ACP nanocomposite in simulated body fluid reaches about 100% in a period of 156 h, which is much slower than that of single-phase calcium phosphate nanostructures. It is expected that the P123/ACP nanocomposite is promising for the application in the controlled delivery of hydrophobic drugs.

**Keywords** Nanocomposite · P123 · Calcium phosphate · Hydrophobic drug · Sustained release

## Introduction

Calcium phosphates exist throughout the body in the form of amorphous calcium phosphate as well as crystalline hydroxyapatite [1]. They have been widely studied as fillers of bony tissues [2, 3], coatings of orthopedic implants [4], fillers of polymer bone cements [5], immovable phases for liquid chromatography of proteins [6], and carriers of cell cultures [7]. Recently, calcium phosphates have been used to provide transfection of DNA and deliver drugs via surface decoration of microparticles [8–13], because calcium phosphates are naturally nontoxic as well as bioresorbable. Calcium phosphates are native to the body, and the problems of metabolism pathway can be greatly reduced compared to other delivery systems [14]. One advantage of calcium phosphates as embedding materials is that they are hydrophilic by nature [15]. Thus, drugs with little solubility in physiological liquids can be delivered through the blood stream to target tissues using calcium phosphates if the hydrophobic drugs can achieve an effective loading capacity.

Amphiphilic block copolymers have recently attracted significant attention in drug and gene delivery research [16–18], especially for hydrophobic drugs [19]. Amphiphilic block copolymers self-associate in aqueous solution, forming a micellar structure with a diameter less than 100 nm. These micelles consist of a hydrophobic core surrounded by a shell of hydrophilic blocks. The core serves as a nonaqueous reservoir for hydrophobic drug, and the shell interacts with the biological environment and serves as a stabilizing interface between the hydrophobic core and the external medium. This characteristic enables both a high loading capacity of hydrophobic drugs and a long circulation time resulting from the steric hindrance due to the presence of a hydrophilic shell and the small scale of polymeric micelles [20]. Besides, this

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characteristic also enables the coating of hydrophilic calcium phosphate on the hydrophilic shell.

Herein, we report the P123/ACP nanocomposite of a typical amphiphilic triblock copolymer P123 (PEO-PPO-PEO) and amorphous calcium phosphate (ACP) as the nanocarrier for hydrophobic drugs, which combines the advantages of both amphiphilic block copolymer and calcium phosphate delivery system. The structure of P123 block copolymer micelles consists of hydrophilic PEO chains attached to the surface of the hydrophobic PPO cores. And the amorphous calcium phosphate is coated on the micelles. A typical hydrophobic drug, ibuprofen, is used to investigate the drug loading and in vitro release behavior in simulated body fluid (SBF). The P123/ACP nanocomposite has higher ibuprofen loading capacity and superior sustained-release property compared with single-phase calcium phosphate delivery system, thus it is promising for the application in hydrophobic drug delivery.

## Materials and Methods

### Preparation of the P123/ACP Nanocomposite

The P123/ACP nanocomposite was prepared as follows: The reaction temperature was kept at room temperature. 0.100 g P123 was dissolved in 40 mL deionized water under magnetic stirring. An amount of 1.5 mL 0.5 M  $\text{CaCl}_2$  aqueous solution was added to the above solution under magnetic stirring. Then, 0.9 mL of 0.5 M  $(\text{NH}_4)_3\text{PO}_4$  aqueous solution was added and magnetically stirred for 30 min. The product was collected by centrifugation and washed by centrifugation-redispersion cycles with deionized water and alcohol three times, respectively.

### Preparation of Single-Phase Calcium Phosphate Nanostructures

The single-phase calcium phosphate nanostructures were prepared as follows: The reaction temperature was kept at 37°C. The pH value of the reaction system was kept at 10. Solution A was prepared by dissolving 1.387 g  $\text{CaCl}_2$  in 75 mL deionized water. Solution B was prepared by dissolving 0.990 g  $(\text{NH}_4)_2\text{HPO}_4$  in 25 mL deionized water. Then, solution B was added to solution A. The product was collected by centrifugation and washed by centrifugation-redispersion cycles with deionized water and alcohol three times, respectively.

### Characterization of Samples

The as-prepared samples were characterized using X-ray powder diffraction (XRD) (Rigaku D/max 2550 V, Cu  $K\alpha$

radiation,  $\lambda = 1.54178 \text{ \AA}$ ), transmission electron microscopy (TEM) (JEOL JEM-2100F), selected-area electron diffraction (SAED), and energy dispersive spectroscopy (EDS). The Fourier transform infrared (FTIR) spectra were taken on a Nexus FTIR spectrometer (Thermo Nicolet, USA). The Brunauer–Emmett–Teller (BET) surface area was measured with an accelerated surface area and porosimetry system (ASAP 2010, USA). The ibuprofen concentrations were analyzed using a UV–Vis spectrophotometer (UV-2300, Techcomp) at a wavelength of 263 nm.

### Drug Loading and In Vitro Drug Release

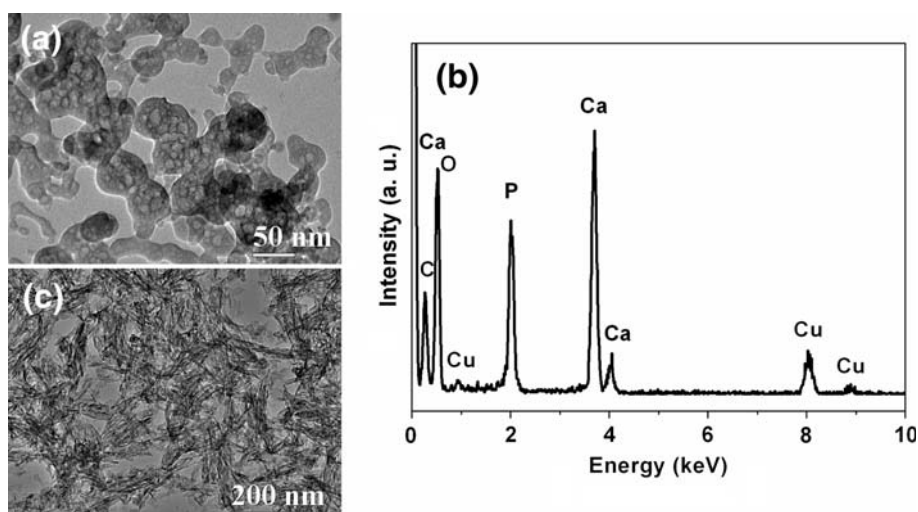
The typical drug loading and in vitro drug release experiments were performed as follows: 1 g of P123/ACP nanocomposite or single-phase calcium phosphate nanostructures was added into 40 mg  $\text{mL}^{-1}$  ibuprofen hexane solution. The suspension was shaken in a sealed vessel at 37°C for 24 h during which the evaporation of hexane was prevented. The sample with loaded drug was separated from the solution, dried, and compacted into disks (each disk 0.3 g) by a pressure of 4 MPa. Each disk was immersed into 200 mL of simulated body fluid (SBF) with pH 7.4 at 37°C with a constant shaking. The shaking device was a desk-type constant-temperature oscillator (THI-92A, China). The release medium (2 mL) was taken out for analysis by UV–Vis absorption spectroscopy at a wavelength of 263 nm at given time intervals and replaced with the same volume of fresh preheated SBF (37°C).

## Results and Discussion

Figure 1a shows the TEM micrograph of the P123/ACP nanocomposite prepared by using an aqueous solution containing  $\text{CaCl}_2$ ,  $(\text{NH}_4)_3\text{PO}_4$ , and P123, which reveals the porous nanostructures with diameters less than 100 nm. EDS analysis (Fig. 1b) shows that the P123/ACP nanocomposite consists of calcium and phosphorus, and the Cu peak is originated from the copper sample holder. SAED as well as XRD pattern indicates that the P123/ACP nanocomposite is amorphous. The BET specific surface area reaches 79.3  $\text{m}^2/\text{g}$ . As a reference, single-phase calcium phosphate nanostructures were also prepared without using P123, which exhibit a bundle-like morphology consisting of nanorods, as shown in Fig. 1c. And SAED and XRD data indicate that the single-phase calcium phosphate nanostructures are also amorphous.

Figure 2 shows the FTIR spectrum of the P123/ACP nanocomposite. The broad strong peak at around 3,427  $\text{cm}^{-1}$  is assigned to the adsorbed water. The characteristic bands of  $\text{CH}_3$ ,  $\text{CH}_2$ , and CH in P123 are located

**Fig. 1** TEM micrograph: (a) and EDS spectrum (b) of the P123/ACP nanocomposite. (c) TEM micrograph of the single-phase calcium phosphate nanostructures with a nanorod morphology



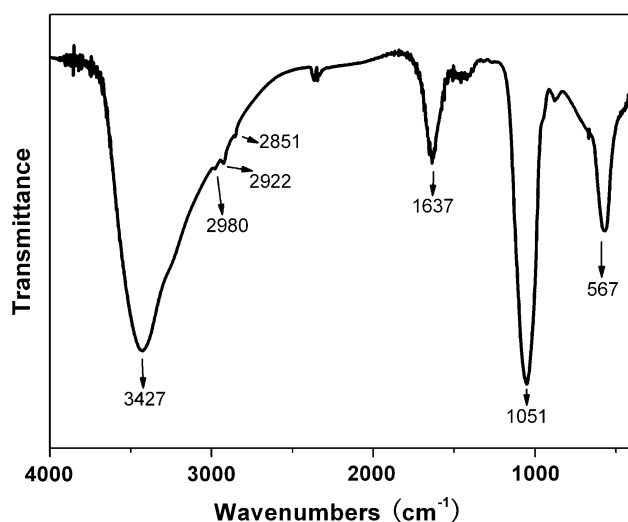
at 2,980, 2,922, and 2,851  $\text{cm}^{-1}$ . The band at 1,637  $\text{cm}^{-1}$  is assigned to the bending mode of the  $\text{OH}^-$  vibration. The intense peaks located at 1,051 and 567  $\text{cm}^{-1}$  are assigned to  $\text{PO}_4^{3-}$ . The characteristic band in the range of 1,100 and 1,000  $\text{cm}^{-1}$  is due to the C–O stretching vibration, which is also a feature of P123; however, it is overlapped with the peak of  $\text{PO}_4^{3-}$ .

It has been reported that P123 self-assembles in water into spherical micelles at ambient temperatures [21]. A P123 micelle consists of a hydrophobic core (PPO block) with a radius of 4.8 nm and a hydrophilic corona (PEO block) with a thickness of 4.6 nm, so that the diameter is around 18 nm [22]. And generally spherical micelles aggregate together [23, 24]. These are consistent with the as-prepared P123/ACP nanocomposite. It is obvious that in the present reaction system, the amorphous calcium phosphate formed in a hydrophilic environment, thus leading to

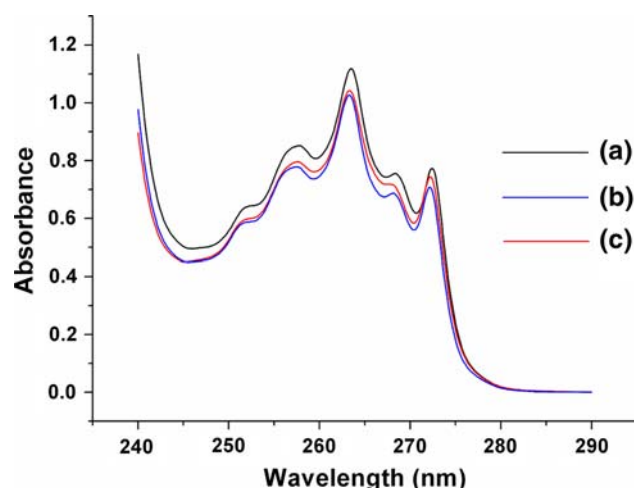
its coating on the hydrophilic corona (PEO block). As displayed in Fig. 1a, the as-prepared samples are composed of aggregated particles. Combining the results obtained from Figs. 1b and 2, one can see that these aggregated particles consist of calcium phosphate and P123. In short, it can be concluded that the P123/ACP nanocomposite formed as following: first, P123 self-assembled into spherical micelles and aggregated together; then amorphous calcium phosphate generated out of the hydrophilic PEO block when  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  were added into the aqueous solution and coated on the micelles.

We explored the possibility of using the P123/ACP nanocomposite as the nanocarrier for drug loading and prolonged drug release. The UV–Vis absorption spectra of the hexane solution containing ibuprofen before and after ibuprofen loading in samples are shown in Fig. 3a–c (each solution was diluted 50 times). One can see that the absorption spectra of ibuprofen in hexane solution show the characteristic absorption peaks of ibuprofen. On the basis of the calculation from the standard concentration calibration curve dependent on the absorbance of ibuprofen at 263 nm, the ibuprofen storage in the P123/ACP nanocomposite is 148 mg/g, obviously higher than the value of the single-phase calcium phosphate nanostructures (118 mg/g), indicating the significant synergic effect of P123 and calcium phosphate on the drug loading.

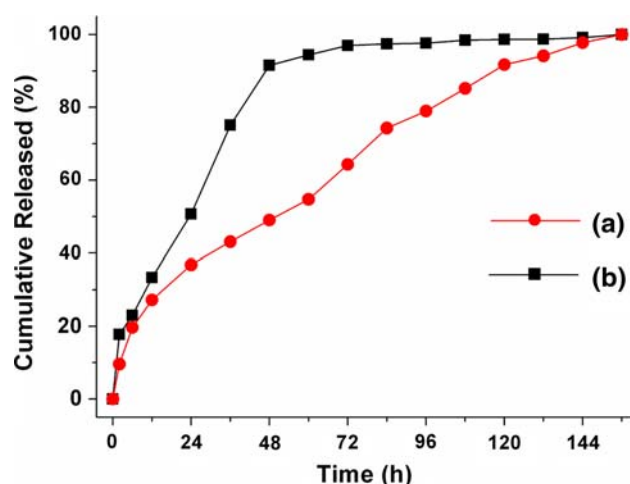
Figure 4 shows the drug release behaviors of the samples in simulated body fluid. The ibuprofen–P123/ACP nanocomposite system exhibits a much lower release rate and sustained release of ibuprofen (Fig. 4a), which can prevent the explosive release of ibuprofen and prolong the drug effect. One can see that about 27% of the loaded drug is released in the first 12 h, then the drug release rate decreases and maintains relatively constant, and reaches a value 49% in 48 h and 100% in 156 h. In contrast, the drug release rate of the ibuprofen–single-phase calcium phosphate system



**Fig. 2** FTIR spectrum of the P123/ACP nanocomposite



**Fig. 3** UV–Vis absorption spectra: (a) the hexane solution containing ibuprofen before ibuprofen loading; (b) the hexane solution containing ibuprofen after ibuprofen loading in the P123/ACP nanocomposite; (c) the hexane solution containing ibuprofen after ibuprofen loading in the single-phase calcium phosphate nanostructures



**Fig. 4** (a) Ibuprofen release curve from the ibuprofen–P123/ACP nanocomposite system in SBF; (b) ibuprofen release curve from ibuprofen–single-phase calcium phosphate system SBF

(Fig. 4b) is much faster, and more than 90% of the loaded drug is released in 48 h. This experimental result strongly reflects the significant synergic effect of P123 and calcium phosphate on the drug release behavior, due to the effect of hydrogen bonds between –OH group of P123 and –COOH group of ibuprofen molecules.

Calcium phosphates, regardless of Ca:P ratio, crystallinity, or crystal phase, are relatively insoluble at physiological pH but have increasing solubility in the acidic environments [1, 15]. This pH-dependent solubility provides an advantage in the controlled drug delivery. And P123 is an important temperature responsive triblock copolymer. Thus, the P123/ACP nanocomposite may also be developed as

both pH- and temperature-responsive drug delivery system. Future work will focus on the pH- and temperature-responsive property of these P123/ACP nanocomposites.

## Conclusions

In summary, we have prepared the P123/ACP nanocomposite with sizes less than 100 nm by using an aqueous solution containing  $\text{CaCl}_2$ ,  $(\text{NH}_4)_3\text{PO}_4$ , and P123 at room temperature. The P123/ACP nanocomposite has been explored as the nanocarrier for hydrophobic drug ibuprofen on the basis of the advantages of both amphiphilic block copolymer and calcium phosphate delivery system. The P123/ACP nanocomposite has a much higher ibuprofen loading capacity (148 mg/g) than the single-phase calcium phosphate nanostructures and has a prolonged drug release behavior in simulated body fluid (156 h), which is much slower than that of single-phase calcium phosphate nanostructures. It is expected that the P123/ACP nanocomposite is promising for the application in the controlled delivery of hydrophobic drugs.

**Acknowledgments** Financial support from the Fund for Nano-Science and Technology from Science and Technology Commission of Shanghai (0852nm05800), the Program of Shanghai Subject Chief Scientist (07XD14031), and the National Natural Science Foundation of China (50772124, 50821004) is gratefully acknowledged.

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